

# **Report on the Coconut Genetic mission to Jamaica**

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## Summary and recommendations

This visit gave us an overview of the progression of Lethal Yellowing in Jamaica as well as of the diversity of coconut germplasm in this country and its response to the disease. We could see that the disease was present in virtually all regions of the island, although unaffected patches still subsist, particularly where the coconut density was lower.

There is a sharp contrast between the situation in the 70' and the present one. The results gathered in the Oleagineux paper by Been in 1981 tended to show that resistance factors were more frequent in germplasm originating from Southeast Asia. This impression was reinforced if we took into account the genetic relations deduced from molecular markers (cf. annex 2). The present situation shows that no cultivar is sufficiently resistant to withstand a high inoculum pressure. This doesn't mean that there are no genetic differences: the susceptible local Tall has virtually disappeared except in the areas where the inoculum pressure was low.

The molecular marker (microsatellites) studies conducted at Cirad show that Panama Talls and the Malayan Yellow Dwarfs used in Jamaica to produce the Maypan hybrid are not absolutely true to type. However, this is probably not the main reason for the recent outbreak of the disease: the level of genetic contamination is insufficient to explain the massive losses observed. On the other hand, both parents of this hybrid variety have a low genetic diversity, and extensively planting the Malayan Yellow Dwarf and the MAYPAN could, in the long run, have contributed to selecting new virulent strains, adapted to these varieties.

In this situation, it is not possible to control the disease by relying on genetic resistance alone. Besides testing new germplasm (preferably of Southeast Asian origin), innovative ways of controlling the inoculum pressure will have to be experimented. We were interested by the experience of M. Black in St Thomas. Although a full scientific proof that the systematic eradication of trees with early symptoms (abnormally high nut fall) is sufficient to keep the disease at a tolerable level, is still to be given, such a measure agrees with common sense. We recommend a scientific assessment of such practices:

- 1) PCR tests should be able to determine whether the trees identified as diseased are really affected.
- 2) The experimental application of such measures should be encouraged in carefully chosen situations.
- 3) These experiments should be followed by the CIB.

Finally, the Fiji Malayan field at Caenwood represents an opportunity to attempt the genetic mapping of genetic factors associated to response to Lethal yellowing. One of the interesting features is that the genetic origins of the parental populations are different. One may thus suspect that favourable genetic factors they present are different. We present the outline of a project in this report.

## Mission Schedule

June 19, 2007 : Flight from Montpellier to Kingston

June 20, 2007 : Stage 1 with Millicent Wallace

Coconut hybrids near a s A UPR 29 7 11 atellite antenna

Michael Black estate (St Thomas)

Plantains garden

Hector's River Hybrid field

Portland (Dwarf coconut hybrids dying on the beach

Fair Prospect

June 21, 2007: Stage 2 with Millicent Wallace

Agualta Vale

Garden near Agualta Vale with suspicious hybrids

Green Castle:

White Rock:

Spring Gardens

Kildare

June 22, 2007: Stage 3 with Wayne Myrie

Barton Isle seed garden

Region of Black river

Night at Montego Bay

June 23, 2007: Stage 4 with Wayne Myrie

Road from Montego Bay to St Ann's Bay

Richmonds farm:

Linstead: Tulloch estate

June 25, 2007: With Millicent Wallace and Dr Basil Been in Kingston

Discussion at the office, exchange of documents

Lunch with the French ambassador

June 26, 2007: Stage 5 with Dr Basil Been

New visit at Spring Garden

Caenwood



Figure 1: Map of Jamaica with the 5 stages of the visit.



## Field visits

### *Michael Black estate (St Thomas)*

The estate covers about 700 acres (65000 trees). The plantation consists in a mixture of Malayan Dwarfs and MAYPAN hybrids. We were received by the owner's son. According to him, Lethal yellowing has been present for 10 to 12 years, but losses don't exceed 50 trees per year in average, which is negligible. He explains this by the adoption of a strict sanitation policy. The main element of this policy is a weekly visit of the trees. As soon as a tree present early nut fall symptoms, it is felled down and burned and quickly replaced by a new one. The owner also managed to obtain the adhesion of his neighbours who apply the same practice. In addition, the soil is maintained clean by the use of herbicides and the trees are fertilized.

We consider this experience as very interesting even if it cannot be proven that the low mortality is the consequence of the practices adopted or that they are sufficient to ensure a permanent protection. In other words, we don't know what would have happened if these practices had not been adopted. We know that the evolution of LY is very variable from a site to another one. It is difficult to understand why sometimes the disease propagate itself very rapidly at one location, but spares another neighbouring location for a very long period of time.

Moreover, the very principle of these practices prohibits the use of a "control" treatment in the same area: such a control would become a possible source of contamination. In addition, the early elimination of the plants might prevent the detection of the phytoplasma and thus make it difficult to assess the intensity of the inoculum pressure.

On the other hand, the policy adopted by M. Black is a very straightforward application of common sense: in order to prevent a rapid propagation of the disease, one should reduce the inoculum to the lowest possible level. It is thus natural to think that it has a beneficial effect on the sanitary state of the effect. Moreover, its soundness seems to be confirmed by work done in Ghana (Philippe *et al.* 2004 - see this paper in appendix 6). The main unknown factor that could influence the outcome is probably the presence or absence of alternative host plants, which would maintain a high inoculum pressure even when diseased coconuts are eradicated.

The only way of confirming or infirming its potential benefits is to repeat this type of experiment in various conditions. To be demonstrative and convincing, these experiments should meet a certain number of constraints:

- They should concern a sufficiently large area: it would be useless for a small farmer to apply this policy if his neighbours don't follow. This type of measure should thus be applied preferentially in large farms or farmer associations. Ideally, the treated area should be isolated from non treated area.
- They should be applied consistently: apparently, the key of success is that the trees are destroyed before a significant number of vectors can acquire the phytoplasma from them and transmit the disease to other trees. Two to 4 visits a month should be adequate.
- The disease should be present at a low level in or in the vicinity of the concerned area. There would be no point in applying this policy in the absence of the disease, but with an increasing proportion of dead or diseased palms, the proportion of infected palms at a latent stage will be too high to make the sanitation measures effective.

- The profitability of the coconut plantation should justify the cost of such a practice. In the case of M. Black's estate, the value added to the product, thank to post-harvest processing, makes the extra costs resulting from the control measure acceptable: The estates markets jelly-nut water, virgin oil and Dwarf coconut seedlings to Florida.

We recommend CIB to identify a limited number of areas meeting the above conditions and to promote experiments of the same type. We think also that CIB should ensure a follow up of

these experiments: are the recommended practice applied correctly? What are the annual losses? Etc...



Figure 2: A hybrid field destroyed by LY at Darlinaford

### *Plantains garden*

We observed King Coconuts obtained from Fair prospect as well as hybrids between the King coconut and the Panama Tall. We also observed Peru Tall and Panama Tall. A special section will be devoted to these cultivars (see Annex 1).

Other cultivars observed were the Sri Lanka Green Dwarf and the Sri Lanka Yellow Dwarf. In the latter, many trees had a hybrid phenotype. We observed the same thing in Sri Lanka. We can also mention the Fiji Malayan, this population results from the first coconut hybrid cross made in Fiji in 1927 by Marechal. The fruits are yellow or sometimes red. This indicates that there has probably been a back cross on the Malayan Dwarf, followed by a selection on the yellow or red colour. The phenotype ranges from a typical Fiji Dwarf -

or Niu Leka - save for the fruit colour to a typical Malayan Dwarf. This accession is a replication of the initial introduction in Fair Prospect.

### *Hector's River Hybrid field (Darlingford) and surroundings*

Figure 2 represents a hybrid field that was completely wiped out by LY. Note that there are still coconuts left uphill while all coconuts on the flat land are dead or dying. Near this place, we passed by a bay where most of the coconuts are dying.

### *Fair Prospect*

Fair Prospect is no longer managed by CIB. However, CIB can still visit it and make observations. LY started to infest this site in 1996. Due to past mortality the coconut density is low. Moreover, upkeep is minimal and circulation is not very easy even with a 4 wheel drive van. We saw many young plants sprouting from unharvested nuts. Among the cultivars that seem to survive, we saw accessions of Bougainville Tall, Sarawak Tall and Fiji Malayan Dwarf whose replication is at Plantain Garden.



The evolution of the disease from 1996 to 2006 is summarized in appendix 1. As always, such results must be taken with caution, since we don't master the inoculation pressure. For example 50% of the "Red Dwarf (local source)" survived, but the "Malaysian Dwarf (Red) ex anchovy estate" was totally destroyed: 131 trees out of 144 were killed by the disease. In our opinion, such a difference cannot be explained by genetic differences within the MYD cultivar.

Near Fair Prospect is the "Erroll Flynn" plantation, which is still unaffected by LY. However, the trees are very yellow. This is probably due to mineral deficiencies. However, it would be a good idea to check for the presence of red palm mites.

### *Agualta Vale*

The Agualta Vale field is one of the oldest Panama Tall fields. It is not affected by the disease. The field presents a mixture of generations (figure 3) and there are a few trees of other varieties outside. These can be sources of genetic contamination for the youngest trees. Some of these young trees were analyzed (see submitted paper in appendix). The analyses made on this field show that 4 out of 11 trees are not pure PNT. Three are F<sub>1</sub> hybrids and one is a first generation back-cross with 75% of JMT genes. Given the small number of coconuts belonging to different cultivars around this field, this suggests that the PNT has a strong tendency to outcrossing.

A few kilometres farther on the way to Green Castle, we observed a few hybrids in a garden. Several of them had yellowing leaves, strongly suggestive of LY.



Figure 3 : Mixture of old and young trees at Aualta Vale



Figure 4: Panama Tall with LY at Green Castle



## *Green Castle*

In the coastal plain, near a pasture, we observed a large number of Dwarfs in poor conditions (nutrition deficiencies). A little farther, a trial testing several hybrid varieties was completely destroyed. Other coconuts from the same variety tested in this trial were planted not far away and are still alive. Some of the trees are hybrids involving the Fiji Dwarf. Finally, we saw the Green Castle PNT field. Here, only 5% are hybrids according to microsatellite studies. A few trees are dying from LY (figure 4).

On the way to Spring Gardens we also saw an untouched field of Panama Tall at White rock (near Kildare)

## *Spring Gardens*



Figure 5: Young Brazilian Green Dwarf with three flowering inflorescences at the same

Spring Gardens is a seed garden which used to be planted with Malaysian Green Dwarfs. All trees died from LY. It has been replanted with Malaysian Green Dwarfs and Brazilian Green Dwarfs about three years ago. The two parts are separated by a brook.

The Brazilian Green Dwarfs are now flowering abundantly. (figure 5). There is also a nursery where two cultivars were grown: Malayan Dwarfs from St. Thomas (M. Black estate) and MAYPANs from Barton Isles.

## *Kildare*



Figure 6: The Niu Leka Dwarf field at Kildare

There is in Kildare a large field with several generations of Niu Leka, locally known as Fiji Dwarf. Although it has a slow growth, it differs from all other Dwarfs and has its origin in Western Polynesia.

It is cross-pollinating, has a bole, a thick, often curved trunk and a large number of leaflets. Moreover, this trait is dominant (the first generation of a cross between a pure Niu Leka and another coconut generally has the Niu Leka phenotype, but there is a disjunction in the further generations).

This cultivar was among those which performed best in presence of LY according to (Been 1981). It is one of the rare cultivars that are still represented at Fair Prospect. Figure 6 clearly shows the successive generation of the Niu Leka. Note the typical thick and the curvature of the trunk. Some of the trees have a typical Tall phenotype, showing evidence of intercrossing.

### ***Barton Isle***

The Barton Isles seed garden was purposefully established outside of the traditional coconut cultivation area. This prevents pollen contamination. This might also reduce the risk of infestation by coconut pests and diseases. The Malayan Yellow Dwarfs that were analyzed in the first paper in annex were all poisoned last year. In fact, the market for yellow coconuts is dramatically shrinking because yellow coconuts are (falsely) associated with Lethal Yellowing. They have been replaced by Malayan Green Dwarfs (from St. Thomas) and a few Brazilian Green Dwarfs.

This replacement is mainly driven by marketing reasons. In principle, it should not be detrimental to the quality of the seeds produced (and, moreover, the Malaysian Yellow Dwarf is no longer considered as resistant). However, seed production using Green Dwarfs requires more skill for the culling of illegitimate (selfed) progenies in nursery. In the case of Yellow or Red Dwarfs, this culling is readily done by eliminating plantlets with a yellow (or red) sprout. This is no longer possible and one can only rely on an assessment of vegetative development in nursery: pure Dwarfs are less vigorous. Another problem is that Green Dwarfs tend to be more heterogeneous than the Yellow Dwarfs, as can be seen with the accession in Côte d'Ivoire. This can be improved by visual selection of mother palms. It would be a good idea to assess the efficiency of such selection by analyzing about 30 individuals with SSR markers (the same experiment that was already done with the "Yard piece" samples from Barton Isle: the samples would correspond to 15 visually true to type individuals and 15 off-type individuals).



Figure 7: Two kinds of « Telephone poles » at Richmond farm

### ***Region of Black river***

LY has been present for one year. We observed a few cases on isolated trees or in small groups. In some of these cases, LY was associated with bud rot.

The prevalence of the disease on the road from Savana to Montego Bay was low. The density of coconuts is low too. Some healthy Local Tall coconuts were seen.

### ***North-West of Jamaica***

On the coastal road from Montego Bay to St Ann's bay, only a few coconuts are remaining.

Some isolated individuals with LY + Bud Rot were present. Figure 7 shows what remains of Richmond Farm, which was planted with as many as 11838 Maypan coconut palms: all died after a few years.

### *Linstead*

Linstead is located on the road from St Ann's Bay to Kingston in the St Catherine parish. We made a rapid visit to Tulloch Estate (owned by M Turner). LY hasn't yet affected this region.

### *Caenwood*

The last day was devoted to the visit of Caenwood, to the north-East of Jamaica. LY is present around this plantation, although patch of unaffected coconuts exist in the surrounding. This visit was an occasion to see two or tree of the Peru Tall from the first generation since introduction. The most interesting part of the visit was however the "Fiji Malayan" field. In effect, this field might be a unique chance of mapping QTLs associated with vegetative development and LY response.



## Results of microsatellite genotyping of Jamaican germplasm

In collaboration with CIB, Cirad analyzed in 2003-2005 the genotype of Panama Talls and Malayan Yellow Dwarfs collected in Jamaica. The aim was to find out whether genetic contaminations could have caused the outbreak of the disease at the end of the last century.

One possible reason for this outbreak was that the planting material didn't correspond to the germplasm that was selected in the seventies. Cirad tested the trueness to type of both parental populations. The answer is:

- 1) there are genetic contaminations in the accessions of both cultivars present in Jamaica
- 2) However, the level of genetic contamination is insufficient to account for the massive losses that have been observed.
- 3) As a result, there must have been a change in the virulence of the pathogen, in the population density or in the behaviour of the vector. These changes may have been induced by changes in the environment, possibly including the massive plantation of the MYD and of the MAYPAN, which have a low genetic diversity. This low diversity might have resulted in the selection of virulent strains.

This research has resulted in two papers that will appear in 2008, although they are already available online. The full text is given in appendices 4 and 5. The first one (Lebrun *et al.* 2008) is entitled **Recent Lethal Yellowing outbreak: why is the Malayan Yellow Dwarf Coconut no longer resistant in Jamaica?** And the second one (Baudouin *et al.* 2008) **The Panama Tall and the Maypan hybrid coconut in Jamaica: Did genetic contamination cause a loss of resistance to Lethal Yellowing?** A third paper dealing with the origin of the Panama Tall is also in preparation.

## Outline of a project for identification of LY related QTLs in coconut.

This project proposal refers to our visit at Caenwood (see above)

### Aims

Identifying QTL markers related to the response to LY in coconut, as well as to morphological traits involved in dwarfism.

### Rationale

The Fiji Malayan at Caenwood are the result of several generations of crossing, starting from the famous cross made by Maréchal in 1928 between the Niu Leka and the Malayan Red Dwarf. Although both parents cultivars are called "Dwarfs", they are very different in term of phenotype as well as of genetic makeup. The usual Dwarfs coconuts, including the Malaysian Dwarfs originated in South-East Asia, are predominantly self-pollinating, have no bole and a reduced number of leaflets, compared to the Tall coconuts. The Niu Leka is a unique cultivar, which originated in Polynesia, is cross pollinating and is similar to Tall coconuts except for its reduced internodes and frond length.

Around 600 trees are planted and families are grouped on the same rows. We saw neither the planting map nor the exact pedigree of these trees. However it is clear that the initial cross was crossed to Malayan Yellow Dwarfs and that only yellow or red trees were selected in the subsequent generations: all the trees are either red or yellow. As a result, the field presents a remarkable disjunction of traits inherited from each parent. There is thus a good chance to reconstruct the family structure.

This field might thus represent a unique chance of identifying QTLs markers related to the response to LY: Since the two kinds of Dwarfs have different origins, we are likely to observe disjunctions at loci involved in the response to LY as well as in marker loci. The large number of individuals and the family structure will facilitate the identification of possible QTLs. In addition to QTLs related to LY, valuable information on the determinism of vegetative development and fruit quality traits will be obtained.

### Constraints

The constraints that may affect the feasibility of the project are inherent to any search for genetic resistance to LY:

- Since we don't master artificial transmission (and since we don't fully understand the natural transmission pattern either) it is difficult to assign a tree its exact status (i.e. resistant or susceptible). The three cultivars (MYD, MRD and NLA) had a good behaviour in the seventies. This fact is particularly well documented in the case of Malayan Dwarfs, which were very abundant and had a very low mortality. Between 1996 and 2006, the MRD had the lowest losses to LY with 50% at Fair Prospect but totally disappeared from Round Hill. The NLA is generally considered as having a good level of resistance in Jamaica and in Florida but has 75% dead trees in 2006 in Fair Prospect.

- Moreover, although the disease is present in the same region, we don't know if and when it will appear at Caenwood itself. We think however, that this should not prevent us from realizing the preliminary stages of the study in order to be prepared when the disease arrives.

In addition

- Although valuable information on the determinism of vegetative development and fruit quality traits can be obtained, yield itself will not be observed due to frequent fruit theft.
- The project needs a high density marker system. The application of DArT ("Diversity Arrays Technology", developed in Australia) markers to germplasm from Vanuatu resulted in a disappointingly low number of useful markers in a coconut DNA representation<sup>1</sup> based on global diversity. For this reason, the project will require the construction of a representation optimized for the Fiji Malayan.

### Project phases

Due to its anticipated duration and to the presence of unknowns inherent to this kind of research, the project will be split into several phases

#### Phase 1: Preliminary observations and DNA collection

When? As soon as possible

Expected output:

- DNA from all individuals,
- An updated map
- Phenotypic data

Activities:

- Establishing a precise plan of the field including information on the relief (A part of the field is on the coastal plain, the other part is hilly)
- Numbering the trees
- Collecting phenotypic data
  - Fruit colour
  - Fruit and nut length and diameter
  - Fruit Component Analysis (if possible)
  - Stem measurements
  - Stem curvature
  - Leaf measurement
- Collecting leaf sample, extracting and storing DNA
- Monitoring of LY presence in the trial and in its surrounding every 3 months

#### Phase 2: Preliminary step of the molecular study

When? Any time between phases 1 and 3

Expected output:

- A high density marker set
- Identification of polymorphic microsatellite markers (to relate the mapping information to the existing map)
- Information on the population structure to be used in phase 4.

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<sup>1</sup> A large set of DNA probes used for DArT markers



#### Activities

- Producing a DArT representation devoted to the Fiji-Malayans
- Genotyping a sub-sample of 50 individuals with 80 microsatellites, 200 AFLP markers and the DArT markers

#### Phase 3: LY data recording

When? As soon as the disease appears at Caenwood and for a 3 year duration period

Expected output:

- Data necessary for QTL identification of LY related traits

#### Activities

- Monthly observation of the LY status of all trees.
- PCR confirmation of the disease in a selected sample.

#### Phase 4: DNA analyzes and QTL mapping

When? When about 50% of the trees are affected by LY

Expected output:

- QTL identification for LY as well as for vegetative and fruit traits.

#### Activities

- Molecular analyzes (SSR, AFLP and DArT ) on 300 individuals. The sampling strategy will involve the family structure, the state (diseased/healthy) and vegetative traits.
- Locationg QTLs. According to the results obtained in phase 2, the approach will combine a LD approach with reconstructed pedigree information.

## References

- Baudouin, L., P. Lebrun, A. Berger, W. Myrie, B. Been *et al.*, 2008 The Panama Tall and the Maypan hybrid coconut in Jamaica: Did genetic contamination cause a loss of resistance to Lethal Yellowing? *Euphytica* published online.
- Been, B. O., 1981 Observations on field resistance to lethal yellowing in coconut varieties and hybrids in Jamaica. *Oléagineux* 36: 9-12.
- Lebrun, P., L. Baudouin, W. Myrie, A. Berger and M. Dollet, 2008 Recent Lethal Yellowing outbreak: why is the Malayan Yellow Dwarf Coconut no longer resistant in Jamaica? *Tree genetics* in press.
- Philippe, R., S. K. Dery and J. Nkansa Poku, 2004 New data on a cultural control method against coconut lethal yellowing in Ghana. *Cord* 20: 21-27.

## ***Annex 1. The Panama and Peru Tall and their origin***

According to molecular markers, the Panama Tall and the Peru Tall are the same variety. This variety seems to descend from a coconut population that existed before Columbus. It was observed on the Pacific coast of the Panama Isthmus. In fact, the Panama/Peru Tall variety is unique among Tall coconuts for its low genetic diversity, which suggest that it resulted from a founder effect: A small number of individuals would have reached America and their descendant would have spread on a large area without genetic contamination from other populations. Due to this small number of founders, the genetic features of this variety would be a highly biased subset of the genetic structure of its population of origin.

We are preparing a paper showing that this origin was probably Southeast Asia. This might be an important clue in the search for genetic factors inducing a reduced susceptibility to Lethal Yellowing. In effect, the Panama Tall (and the Malayan Yellow Dwarf) survived up to recently in Jamaica, when other cultivars had been dying for a long period. Even though their resistance factors appear to have been overcome, related germplasm from Southeast Asia is the most likely to present similar genes, that could remain (at least for a certain period) resistant.

M. Been provided us a very interesting internal CIB document about the origin of the Peru Tall: they were shipped from Sullana, in the northern part of Peru (not really at the border, contrarily to what is said in the document!). In the same paragraph, the document mentions the case of coconuts imported from Esmeralda (Ecuador) to Cayman Brac, claimed to be resistant to LY in 1967. These coconuts had a similar fruit composition as the "San Blas" (a.k.a. the Panama Tall).



## Annex 2. Response to LY of cultivars from Southeast Asian origin

We made statistical analyses on the paper published by Been 1981 in *Oleagineux* in order to estimate the "breeding value" of the different cultivars mentioned in the paper. This analysis uses the generalized linear model and takes into account the results of both hybrid and pure cultivars (the predicted performance of a hybrid is supposed to be the average between those of its parents). It takes also into account the variation between environments. The results can be summarized by figure 8.

Several cultivars have a Southeast Asian origin:

All Dwarf (yellow and orange on the graph) had a Southeast Asian ancestor, but the "Pacific type Dwarf" (orange) have been recombined with local varieties in Papua New Guinea.

Then we have the Southeast Asian Talls themselves, (green), the Panama and Peru Talls (pink). Other varieties probably share many genes with Southeast Asian cultivars: The Micronesian cultivars (pale blue) and the Mozambique Tall (pale pink), which has been influenced by the consequences of Austronesian migrations to Madagascar. The other groups are the Melanesian (brown), Polynesian (dark blue) and "Indo-Atlantic cultivars (red).

Most of the cultivars of those groups have predicted mortality rates above 50% (with 3 exceptions) In contrast, all cultivars of direct or indirect Southeast Asian origin had less than 50% predicted losses to LY (in 1981!). The only exception is the Rangiroa Dwarf which appears to have also genes from PNG. Note: It has to be noted that most of these cultivars have died at present. A balance of the 96-2006 period is given in appendix 3

These results suggest that Southeast Asian coconuts have more resistance - or reduced susceptibility - factors than those from elsewhere in the world

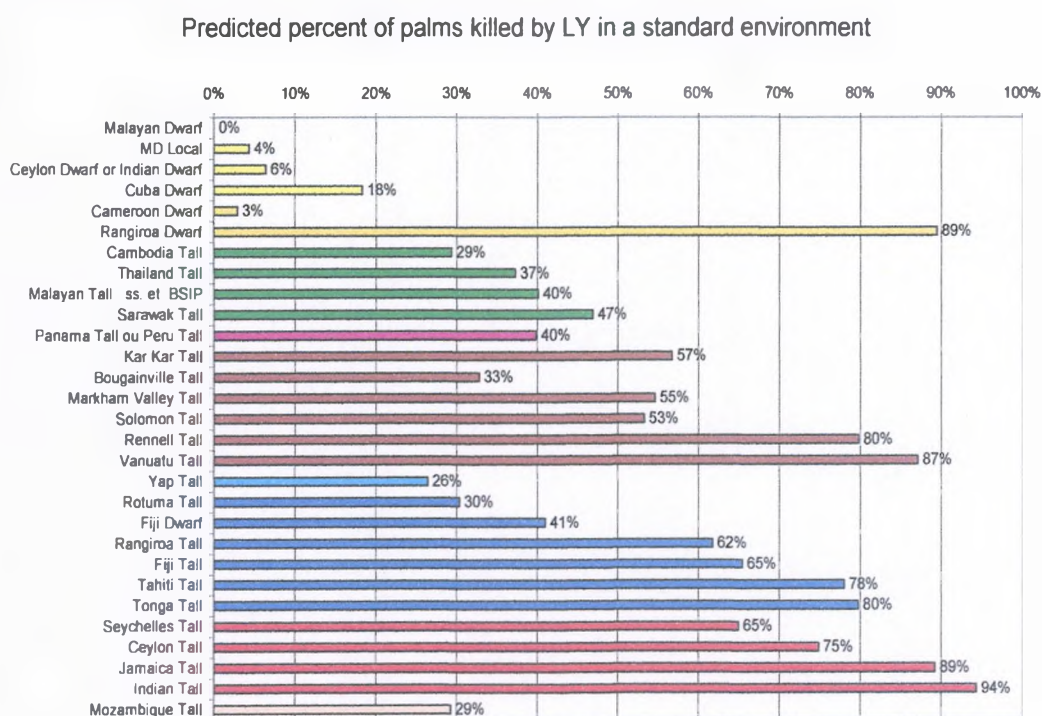


Figure 8: Breeding value of 29 cultivars (as to 1981)

**Annex 3. Evolution of the disease between 1996 and 2006 in Fair Prospect modified after data kindly provided by M. Wallace**

Part 1: Accessions with remaining trees

Variety	Present in 1996	Present in 2006	LY mortality	Other causes
MRD (Malaysian Red Dwarf) <sup>1</sup>	22	11	11	0
SKT (Sarawak Tall)	109	20	76	13
Maypan	14	4	10	0
NLAD (Niu Leka)	31	7	23	1
PNT x BSIT (San Blas x BSIP)	17	4	13	0
BOT (Bougainville Tall)	56	6	46	4
MGD x CRD (Malayan GD x Cameroon RD)	12	2	10	0
MGD x JMT	12	2	10	0
MYD x CRD (Malayan YD x Cameroon RD)	20	3	17	0
FMOD x JMT Fiji Malayan Orange x Jamaica Tall	20	2	17	1
MLT Malayan Tall (including FMS and BSIP)	32	3	27	2
FJT (Fiji Tall)	8	1	7	0
MPET (Maphrao Thailand Tall)	230	20	202	8
JMT x MYD (Jamaica T x Malayan YD)	26	3	23	0
MYD (Malayan Yellow Dwarfs - selfing)	14	1	13	0
JMT x MGD (Jamaica Tall x Malayan Green Dwarf)	14	1	13	0

<sup>1</sup>Compare with Malayan Dwarf (Red) ex Anchovy estate below

Part 2 : Accessions that have disappeared

Variety	Present in 1996	Present in 2006	LY mortality	Other causes
Malayan Dwarf (Red) ex Anchovy estate	144	0	131	13
Chowghat Green Dwarf	9	0	3	6 *
Sri Lanka Green Dwarf	17	0	12	5 *
Sri Lanka Yellow Dwarf	17	0	14	3 *
King coconut	7	0	7	0
Cambodia Tall	79	0	79	0
Kar Kar Tall	37	0	36	1
Markham Valley Tall	14	0	13	1
Rennell Isl. Tall	3	0	3	0
Vanuatu (New Hebrides) Tall	3	0	2	1 *
Rotuman Tall	3	0	3	0
Rangiroa Tall	5	0	5	0
Tahiti Tall	8	0	8	0
Western Samoa Tall	11	0	11	0
San Blas (Red) x Cameroon Red Dwarf	17	0	14	3
Malayan Yellow Dwarf x Mozambique Tall	4	0	4	0
Malayan Yellow Dwarf x Cambodia Tall	2	0	2	0
Malayan Green Dwarf x Tahiti Tall	5	0	5	0
Yellow Dwarf x Rennell Tall	11	0	11	0
Yellow + Red Dwarfs x BSIP	12	0	12	0
Yellow Dwarfs x Jamaica Tall	22	0	22	0
Jamaica Tall x Red Dwarf (exotic)	17	0	16	1
Jamaica Tall x Red Dwarf (local)	4	0	4	0
Jamaica Tall x Mozambique Tall	3	0	3	0
Jamaica Tall x San Blas (Green)	6	0	6	0
Jamaica Tall x Cambodia Tall	3	0	3	0
Jamaica Tall x Tahiti Tall	2	0	2	0
Jamaica Tall x San Blas (red)	9	0	7	2 *
San Blas (Red) x Jamaica Tall	6	0	6	0
San Blas (Green) x Jamaica Tall	6	0	6	0
San Blas x Rennell Tall	6	0	6	0
San Blas x Tahiti Tal	4	0	4	0
Fiji Malayan x Fiji Malayan	17	0	14	3
Total	1150	90	992	68

Note some differences with the initial tables. We assumed that the "mortality for other causes" was the difference between the number of trees in 1996 and the number of trees that died from LY...



## APPENDICES

- Appendix 4: Paper in press in *Tree genetics and genomes*
- Appendix 5: Paper in press in *Euphytica*
- Appendix 6: Paper published in *Cord*

## Appendix 4

## Recent lethal yellowing outbreak: why is the Malayan Yellow Dwarf Coconut no longer resistant in Jamaica?

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**Abstract** In Jamaica, the Maypan, a hybrid of Malayan Yellow Dwarf (MYD) and Panama Tall coconut, previously considered highly resistant, is currently being devastated by an epidemic outbreak of lethal yellowing disease. There are several possible causes for this change. In this study, we checked that affected planting material in Jamaica is genetically the same as the material shown to be resistant. We compared the deoxyribonucleic acid (DNA) of MYD sampled in four locations in Jamaica with a reference DNA of the same cultivar collected in five different countries. The results of our analyses showed more variation at 34 simple sequence repeat loci in Jamaica than in the rest of the world providing clear evidence for the presence of about 16% of alleles that do not match the usual typical MYD genotype. These alleles appear to have already been present in the introduced germplasm. This rules out a possible cause of the new outbreak: The observed hetero-

geneity may have caused some loss of resistance but is insufficient to explain a massive outbreak of the disease.

**Keywords** Lethal yellowing · Phytoplasma · SSR · Cultivar identification · Molecular markers · Disease resistance

### Introduction

A new outbreak of lethal yellowing (LY) is devastating the coconut palm grove in Jamaica. Affected varieties include the Malayan Yellow Dwarf (MYD) and its hybrid with the Panama Tall or Maypan, which had been recommended as resistant. This raises several questions:

- Did the virulence of the pathogen change through mutation and/or selection of virulent types?
- Did a change occur in the nature or in the behaviour of the vector?
- Is the planting material that is affected today the same as the one that was declared resistant two decades earlier?
- Are the populations used in Jamaica representative of the cultivar they are supposed to belong to?

Answering these questions is essential for establishing a sound control strategy. Molecular markers can now answer the last two questions. We propose to show how assignment methods can be used to assess the trueness-to-type of genetic material used in genetic trials and in seed production. We will concentrate here on the MYD found at different locations in Jamaica and how it differs from those found in other countries.

MYD is a genetically uniform cultivar because it has a strong tendency to self-pollinate under natural conditions.

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The resulting natural tendency of the MYD towards homozygosity is further increased by human selection based on the distinctive yellow colour of the sprout. Bourdeix (1988) showed that this colour is determined by two recessive genes. Selection based on colour is very efficient in discarding out-crossed seedlings.

Jamaican MYD represents an exception among MYDs in that it is visually more heterogeneous than populations from other countries (Fig. 1).

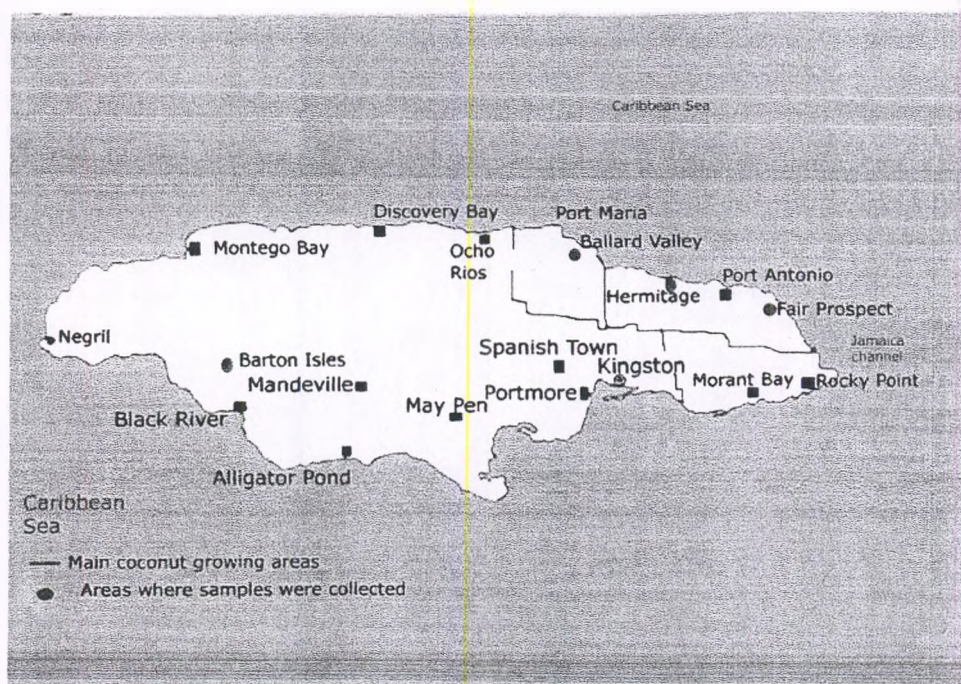
This cultivar was introduced to Jamaica at different periods. The seeds never came directly from Malaysia. Whitehead 1966 reported that first importations of MYD and Malayan Red Dwarf (MRD) from Miami took place in 1939 and in 1940. Only four individuals survived and had progenies living in 1966. In the second case, MYD was introduced from Sri Lanka or Malaysia via Trinidad. There were two survivors and 15,000 progenies existed in the West End in 1966. However, the majority of the MYD present on the island resulted from later importations from Santa Lucia after serious hurricanes in 1944 and in 1951. Some 70,000 Malayan Dwarfs were imported before 1951. The initial source material in Santa Lucia consisted in 18 Malayan Dwarfs (six Green, six Red and six Yellow), imported from Malaysia (then the Federated Malay States).

Hurricanes were not the only cause of losses of coconut trees. LY, a coconut palm disease, is currently devastating the Caribbean region, including Jamaica. The first reports of the presence of the disease in Jamaica appeared in 1884 (Coconut Industry Board 1971). Mycoplasma-like organisms (now known as phytoplasma) were observed by

electron microscopy and found specifically associated with LY syndromes in Florida and in Jamaica (Beakbane et al. 1972; Heinze et al. 1972a, b; Plavšić-Banjac et al. 1972). In 1983, Howard et al. showed that *Mindus crudus*, a plant hopper, is the vector of the disease in Florida. LY took 10 years, from 1961 to 1970, to invade all of Jamaica. By 1980, the disease had been responsible for the death of more than 7 million coconut palms in Jamaica. Malayan Dwarf, then Maypan (cross of Panama Tall and Malayan Dwarf), were replanted across the island because they were considered at that time as the most resistant varieties (Been 1981). However, Howard et al. 1987 reported losses because of LY in the MYD as early as 1987. At various locations along the coastal areas of the northern region, mortality levels among stands of MYD and Maypan were observed to be consistently higher than anticipated (Myrie 2005). This pattern continued into the 1990s, and in certain locations, the disease has started to move away from the coastal areas. The infection rate of the disease was increasing significantly. It was clear that LY re-emerged as the single most important plant disease affecting the coconut industry of Jamaica. In total, this new outbreak destroyed 1.3 million coconut trees.

Among the possible reasons cited to explain this apparent change of behaviour is that the MYD dying from the disease could differ genetically from those that were tested in the resistance trials. To check this hypothesis, we examined the diversity of the MYD presently found in Jamaica and compared it with degree of variation observed in the same cultivar in various countries.

**Fig. 1** Map of Jamaica showing the major coconut growing areas and locations where samples were collected





## Materials and methods

### International reference for MYD

The reference samples we used to characterize the MYD were collected in five different countries. The 20 individuals from Côte d'Ivoire were taken from two accessions: One was imported from Malaysia (13 individuals) and the other one from Ghana (seven individuals) and is also known as the Ghana Yellow Dwarf. The other countries were the Philippines, India, Mexico and Malaysia itself (Table 3 below).

Eleven MRD and five Malayan Green Dwarf (MGD) samples from the Marc Delorme Research station were also analyzed for the same loci.

### Jamaican MYD germplasm

The Jamaican MYD germplasm came from four locations (Table 1 and Fig. 2). The samples were collected between February 2, 2005 and May 26, 2005 at the location shown on the map. Among the samples from Barton Isles, we distinguished two sets, which were visually identified as "true to type" or "off type" based on the presence or absence of a bole and on height growth.

### Microsatellite markers

The molecular markers used for this study were 14 microsatellite (simple sequence repeat, SSR) markers from the "microsatellite kit for coconut cultivar identification" (Baudouin and Lebrun 2002; Lebrun et al. 2005) and 20 additional markers. The selection criteria for these markers were that they should be easy to implement and to score. Table 2 lists some details on the 34 SSR markers.

### SSR genotyping

SSR analyses were performed on an automatic sequencer Li-Cor IR2 (Lincoln, NE). All technical conditions are described in Baudouin et al. (2005).



Fig. 2 An off-type coconut MYD with a bole at the base of the trunk. Trees in the background have the typical phenotype (without bole)

### Statistical analyses

We grouped the genotypes at each locus into four categories: (A) homozygote for the most frequent allele (described below as "the typical MYD genotype"), (B) homozygote for another allele, (C) heterozygote possessing the most frequent allele and (D) heterozygote for two rare alleles (see Table 3). This made it possible to calculate two further parameters: observed heterozygosity as  $(C+D)/N$  and occurrence of non-typical alleles as  $1-(A+C)/N$  with  $A$ ,  $B$ ,  $C$  and  $D$  being the number of genotypes in each category and  $N=A+B+C+D$  being the total number of genes scored. In addition, we estimated Nei's diversity index  $h=2n(1-\sum x_i^2)/(2n-1)$  (Nei 1978) where  $x_i$  is the frequency of the  $i$ th allele of the considered locus and  $n$  is the number of individuals.

## Results

### Reference samples

Excluding Jamaica, we scored 55 MYD individuals at 34 loci. Of the  $55 \times 34 \times 2 = 3,740$  resulting allelic observations,

**Table 1** list of samples studied in Jamaica

Place of collection	Planting date	Code	Visual appearance	Number
Fair Prospect	Around 1960	FP	n.a.	5
Barton Isles	1973–1974	BI	n.a.	31
Barton Isles, Yard Piece	1973–1974	YPt	True-to-type (Yard Piece)	7
Barton Isles, Yard Piece	1973–1974	YPo	Off-type (Yard Piece)	5
Hermitage	Around 1960	H	n.a.	11
Ballards Valley	Around 1960	BV	n.a.	10

**Table 2** List of SSR markers used for assessing the trueness-to-type of MYD individuals

Number	SSR clone	5'...3' Forward primer	5'...3' Reverse primer	Linkage group <sup>a</sup>	EMBL acces. number	Detection power <sup>b</sup>	Size of MYD (or MRD) typical allele (bp) <sup>c</sup>
Markers from the microsatellite kit							
1	CnCir A3	AATCTAAATCTACGAAAGCA	AATAATGTGAAAAAGCAAAG	3	AJ458309	21	240
2	CnCir A9	AATGTTTGTGTCTTTGTGCGTGTGT	TCCTTATTTTCTTCCCCTTCCTCA		AJ458310	6	89
3	CnCir B6	GAGTGTGTGAGCCAGCAT	ATTGTTACAGTCCTTCCA		AJ458311	39	202
4	CnCir B12	GCTCTTCAGTCTTTCTCAA	CTGTATGCCAATTTTCTA		AJ458312	46	169
5	CnCir C3'	AGAAAGCTGAGAGGGAGATT	GTGGGGCATGAAAAGTAAC		AJ458313	15	174
6	CnCir C7	ATAGCATATGGTTTCCT	TGCTCCAGCGTTCATCTA		AJ458314	17	161–(163)
7	CnCir C12	ATACCACAGGCTAACAT	AACCAGAGACATTGAA		AJ458315	22	183
8	CnCir E2	TCGCTGATGAATGCTTGCT	GGGGCTGAGGGATAAACC		AJ458316	3	135
9	CnCir E10	TTGGGTTCCATTCTTCTCTCATC	GCTCTTTAGGGTTCGCTTTCCTAG		AJ458317	13	238–(232)
10	CnCir E12	TCACGCAAAAAGATAAAACC	ATGGAGATGGAAAGAAAGG		AJ458318	3	164
11	CnCir F2	GGTCTCCTCTCCCTCCTTATCTA	CGACGACCCAAAACCTGAACAC		AJ458319	13	205
12	CnCir G11	AATATCTCAAAAATCATCGAAAG	TCATCCCACACCCTCCTCT		AJ458320	33	194
13	CnCir H4'	TTAGATCTCCTCCCAAAG	ATCGAAAGAACAGTCACG		AJ458321	23	230
14	CnCir H7	GAGATGGCATAACACCTA	TGCTGAAGCAAAAGAGTA		AJ458322	5	139
Additional markers							
15	CnCir A4"	GTTGGTTACTGGAAATCTT	CATGACATACGGACTAGC	3	AJ865089	44	200
16	CnCir B3	CATCTTGCTTTTACCATCC	AATACTGTGCGGTTTGTCTT		AM113713	10	293
17	CnCir B4'	TTTCATTCCAAGAGCCTAC	TTTCAAGCATCATTTCAACT		AJ865091	8	196
18	CnCir B11'	TCTGCATCCCTTCTTTATTA	TCTGCATCCCTTCTTTATTA	10	AJ865093	34	110
19	CnCir C5	ACCAACAAAGCCAGAGC	GCAGCCACTACCTAAAAAG		AJ865094	17	132
20	CnCir C9	CAGAAAGGAGAAAGGAAAT	CTACGATAGAGGAATGAGC	4	AJ865095	25	214
21	CnCir C11	TGTTATTTTGTTATTTTCAGG	TCACCATCTTCTCAGTTTC	4	AJ865096	10	221
22	CnCir D1	GGGAGGGGAGCGAGACTATG	AATTCAGGCCAACACCAGACC		AJ865097	15	204
23	CnCir D8	GCTCTTGATGTGGCTGCT	AGGCGTGTGTGAGATTGTGA	4	AJ865098	44	251–(245)
24	CnCir E1	CTTGTTATGTGCTTTGTTG	CTGAGACCCTGTTGATGT		AJ865099	8	227
25	CnCir E4'	GCATGGTATTCGGATTTG	ATGGTTCAGATTGGACAGT	5	AJ865100	3	187
26	CnCir E7	AACTACAACAAGAAACACG	AGAGAAGGGAAAAGGATA		AJ865101	55	216
27	CnCir E11	CAGAAACAGCCAATCAAGCAATA	ATCCATAATAGCCACTCAACAAAA	4	AJ865102	25	200–(196)
28	CnCir F3'	CCCTACTACTCCCTCAT	TGCCTAGTCAATCATAC		AJ865103	22	172
29	CnCir G4	AGTATAGTCACGCCAGAAAA	AAACCCATAACCAGCAAG	5	AJ865104	22	168
30	CnCir H11	TCATTAGAGGACAAAAGTT	TAAAAATTCATAAAGGTAAAA		AJ865105	25	173–(193)
31	CnCir I4	TCCTAGTGCTTATGCTTGAC	TTGATGGTTTGATGTTGAA	15	AJ865106	0	285
32	CnCir J2	CCATTGTCTATTGTTATTTTG	GTCACCATCTTCTCAGTTTC	15	AJ865107	6	232
33	CnCir J10	GAGGGTATGGTGTGCTTG	ATCCTTCATGTGGCTCGTC	10	AJ865108	6	150
34	CnCir K8	CCAGACATGAAACAAACAA	CATGGCACATAGGAAGAAC	13	AJ865114	20	162

<sup>a</sup> See Baudouin et al. 2005<sup>b</sup> Number of occurrences of "outsider" alleles detected in the Jamaican accessions using the locus<sup>c</sup> The typical allele size for the MRD is given in parentheses when it differs from the MYD allele

**Table 3** Summary results of genotyping of the MYD in reference samples and in Jamaica

Collected country	Number	Homozygotes (%)		Heterozygotes (%)		Heterozygosity rate (%) E	Percentage of non-typical alleles (%) F
		For MYD alleles A	For non-typical alleles B	With 1 MYD allele C	With 2 non-typical alleles D		
<b>Côte d'Ivoire</b>	20	100.0	0.0	0.0	0.0	0.0	0.0
<b>Mexico</b>	3	100.0	0.0	0.0	0.0	0.0	0.0
<b>Philippines</b>	10	99.4	0.6	0.0	0.0	0.0	0.6
<b>India</b>	10	92.6	2.4	5.1	0.0	5.1	5.0
	9	97.1	2.6	0.4	0.0	0.4	2.8
<b>Malaysia</b>	12	99.7	0.0	0.3	0.0	0.3	0.1
Average							
All data	<b>55</b>	<b>98.5</b>	<b>0.5</b>	<b>1.0</b>	<b>0.0</b>	<b>0.96</b>	<b>1.10</b>
Without F <sub>1</sub> hybrid	54	99.4	0.5	0.1	0.0	0.12	0.58
<b>Jamaica</b>							
Fair Prospect (FP)	5	86	5	9	0	9	14
Barton Isles (BI)	31	86	9	4	1	5	13
True type (Ypt)	7	94	3	3	0	3	6
Off type (Ypo)	5	76	8	15	2	17	23
Hermitage (H)	11	74	9	15	1	17	25
Ballard Valley (BV)	10	72	13	13	3	16	27
Average							
All data	<b>69</b>	<b>82</b>	<b>9</b>	<b>8</b>	<b>1</b>	<b>9</b>	<b>17</b>
Without F <sub>1</sub> hybrids	67	84	9	6	1	7	16

Average values are given in bold. Figures in italics are averages obtained after removing the obvious hybrids

3,536 were scorable, and 3,501 corresponded to the most frequent allele at their respective locus, i.e. the "typical allele" of the MYD. Of the remaining 35 occurrences of rare alleles, 15 were found at heterozygous loci in a single individual from India. This individual was thus not a MYD but a hybrid (possibly a F<sub>1</sub> hybrid or a backcross with an undetermined local cultivar). Five individuals were homozygous for a rare allele at a single locus and two other at two loci. Finally, two individuals heterozygous at a single locus accounted for the remaining cases.

CnCirE7 and CnCirC7 were the two most polymorphic markers. CnCirE7 deviated from the typical genotype in five homozygous individuals. Three of the five individuals came from India and shared the same rare allele. The two remaining individuals were from the Philippines and had a rare allele in common, different from the one observed in

the Indian germplasm. At locus CnCirC7, the three Indian variants were also homozygous for the same allele, suggesting that the variant individuals from the same country are related.

Among the 11 MRD samples, eight had exactly the same genotype, differing from the MYD genotype at only five loci. One had the typical MRD genotype except that it had the MYD allele at one locus. The remaining two differed from the typical genotype at, respectively, six and seven loci. The genotype of the MGD was more complex: The most frequent allele was generally the same as in the MYD, but polymorphism was found at 21 loci. Moreover, observed heterozygosity was 10%. It is worth noting that the MGD is much less homogeneous phenotypically than either the MYD or the MRD. The tendency to self-pollinate is less pronounced in the MGD than in the other Malayan Dwarfs.

**Table 4** Status of the Jamaican MYD samples according to their microsatellite genotype

	Number	True to type	2 non-typical alleles or less	F1 hybrids	2e generation hybrids	Others
Fair Prospect (FP)	5	0	0	0	1	4
Barton Isles (BI)	31	9	2	0	1	19
True type (YP) (Ypt)	7	2	3	0	0	2
Offtype (YP) (Ypo)	5	0	0	0	2	3
Hermitage (H)	11	0	1	1	2	7
Ballard Valley (BV)	10	0	1	1	1	7
Overall	69	11	7	2	7	42



## Jamaican MYD

Among the 69 individuals collected in Jamaica, only ten had the typical MYD genotype at all loci; six further individuals differed at no more than two loci. On the other hand, two individuals were obviously recent hybrids as suggested by the high level of heterozygosity (>50%) and the large number non-typical alleles observed. One of the hybrids (from Ballard) is likely a  $F_1$  hybrid with the Panama Tall while the other (from Hermitage) appears to be a hybrid with the local cultivar Jamaica Tall. Seven more individuals had a heterozygosity rate between 0.35 and 0.20 and were possible backcrosses or  $F_2$  hybrids. The majority of the analyzed individuals did not fall into the previous categories and had several non-typical alleles (presumably “outsiders”) and a moderate rate of heterozygosity (Table 4).

The percentage of non-typical MYD alleles per individual varied from 0 to 74% (average=17%). Excluding the apparent  $F_1$  hybrids, the heterozygosity rate was moderate (7%) and substantially lower than the Nei's diversity index (0.22), yielding a  $F_{IS}$  close to 0.66. This indicates a high rate of inbreeding and suggests that the MYD from Jamaica is highly self-pollinating, like any other Dwarf (Table 3). If we consider samples separately, the sample with the fewest non-typical MYD alleles (6%) and the lowest observed heterozygosity (3%) was the true-to-type Yard Piece sample from Yard Piece. At the other end of the scale, the Ballards Valley, Hermitage and off-type Yard Piece samples had about 25% of non-typical MYD alleles and about 17% observed heterozygosity. Finally, comparing the three samples from Barton Isles (Barton Isles, true-to-type Yard Piece and off-type Yard Piece), the visually true-to-type individuals (true-to-type Yard Piece) had fewer non-typical MYD alleles than either the “off-types” (off-type Yard Piece) or random sample (Barton Isles). They were however not all “pure” MYD: The percentage of non-typical alleles was still ten times larger than in the reference samples.

## Detection power of the markers

The low degree of polymorphism expected from this highly self-pollinating cultivar required a large marker set. For example, it would not have been possible to detect heterogeneity in the Philippines with the 14 markers of the “coconut cultivar identification kit” alone. In this case, the whole 34-marker set proved to be necessary. However, we could reduce this number in further studies based on the experience acquired here. In effect, the markers used were not equally able to detect polymorphism. The number of non-typical MYD alleles detected in Jamaica (Table 2, seventh column) varied from 0 to 55 (or 40%) according to the marker, and 50% of this polymorphism was detected with only nine markers (CnCirE7, CnCirB12, CnCirA4”,

CnCirD8, CnCirB6, CnCirB11', CnCirG11, CnCirE11 and CnCirH11). To detect 90% of the heterogeneity, 13 additional markers would be necessary, thus saving 35% of the genotyping effort required. Finally, we note that although they were selected under somewhat different conditions, there is virtually no difference regarding polymorphism detection between the 14 usual and the 20 additional markers: The average value is 18.5 and 20, respectively.

## Conclusion

The issue of varietal identity in the MYD in Jamaica is critical because this cultivar was claimed to be resistant to LY and has been extensively used to control the disease as a pure variety or as the female parent of the Maypan. Obviously, such a claim is meaningful only if the distributed planting material corresponds to what was tested. This had to be questioned because both the MYD and the Maypan are presently being devastated by the disease. The present study shows that the Jamaican MYD is only partially true to type. This heterogeneity may have some adverse effect on its degree of resistance but is not sufficient to account for the “massive” losses described by Broschat et al. 2002. Moreover, heterogeneity in the Jamaican MYD is not a recent phenomenon, as the oldest stands (Hermitage and Ballard Valley) are among the most heterogeneous. We are thus forced to conclude that MYD itself is no longer (if it has ever been) fully resistant to the current strains of LY phytoplasma.

We obtained for the first time a rough but quantitative evaluation of the normal degree of variation in a Dwarf coconut: The rates of non-typical alleles and of heterozygosity are, respectively, close to 0.5 and 0.1% heterozygous loci in the MYD. These values are rather conservative if we remark that accessions from Côte d'Ivoire and Mexico were identical at all 34 loci. This confirms that both its self-pollinating habit and human selection tend to make the MYD a highly uniform cultivar (Sangaré et al. 1978; Sangaré 1981). The presence of a small proportion of non-typical alleles can be explained by the fact that they were not yet eliminated by the selfing process. In theory, their frequency is reduced by half at each subsequent generation (Falconer and Mackay 1996). In addition to mutation, occasional crosses with closely related genotypes may also contribute to diversity, provided that their progenies are phenotypically close enough to the MYD.

Polymorphism in Jamaica is far beyond the normal range for the MYD. The rate of non-typical alleles and of heterozygotes were, respectively, 7 and 16% (excluding the two obviously hybrid genotypes). Only 16% of the Jamaican genotypes were true to type (26% if we allow for



two non-typical alleles). The account of MYD introduction by Whitehead 1966 supports the hypothesis that heterogeneities in the MYD have been there since the beginning: The most likely origin of the majority of the tested samples is the introduction from Santa Lucia, which comprised all three Malayan Dwarfs. In addition, the MGD is more heterogeneous than the other two, partly because the nursery selection criterion based on colour is no longer available. It is thus more likely to harbour alleles originating from another cultivar because of occasional intercrossing. Crosses among these initial introductions have probably created the observed diversity.

The structure of diversity in the Jamaican MYD seems to illustrate a possible mechanism of evolution in Dwarf coconuts. In effect, we observed a combination of a rather high proportion of non-MYD alleles with a moderate degree of heterozygosity. Although they are not all true to type, most Jamaican MYDs may be considered as "normal" Dwarfs as far as their reproductive behaviour is concerned. They are presently slowly returning to a homozygous state, thus producing a bundle of closely related pure lines, differing by the varying fraction of non-MYD alleles they incorporated. During this process, natural selection is at work: Besides LY, the strongest selection pressure is probably due to hurricanes, to which the MYD is highly susceptible. This probably leads to a selection in favour of non-MYD alleles in the chromosomal regions that are involved with a strong rooting system and reduced precocity. Finally, visual selection of true-to-type MYDs was probably less stringent than elsewhere in the world owing to the lack of a "pure" reference population. The observations made at Barton Isles confirm that visual selection, although effective, is insufficient to restore the original MYD genotype.

For germplasm conservation and seed production, it is important to keep the original MYD germplasm in Jamaica. This could be done through new importation; however, an acceptable method would be to collect nuts from the (molecularly as well as visually) true-to-type material identified at Yard Piece (Barton Isle). Combining this collection with nursery selection and emasculation of the off types will ensure a high degree of purity. This is not to say that the diversity that was involuntarily created in the Jamaican MYD should be discarded right away. Its interest lies in the fact that it is partly fixed, thus making family-based selection easier. As an example, such selection, monitored by molecular genotyping, could rapidly arrive at "improved" MYD lines, showing improved resistance to hurricanes.

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## **Appendix 5**

# The Panama Tall and the Maypan hybrid coconut in Jamaica: did genetic contamination cause a loss of resistance to Lethal Yellowing?

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**Abstract** We applied Bayesian population assignment methods to assess the trueness to type of four populations of the coconut cultivar Panama Tall (PNT) located in Jamaica and found that two of them presented a high percentage of off-types, while genetic contamination was low in the two others. The PNT is the pollen parent of the MAYPAN hybrid, which used to be planted in Jamaica to control an epidemic disease: Lethal Yellowing. The main source of contamination was the susceptible Jamaica Tall, thus increasing the susceptibility in the resulting MAYPAN progeny. The incidence of genetic contamination seems however to be insufficient to be the only cause of the latest outbreak of the disease. Neither the MAYPAN nor its parents can be said resistant in the present context of Jamaica.

**Keywords** Coconut · Lethal Yellowing ·  
Phytoplasma · Genetic control · Microsatellites

## Abbreviations

SMD-CNRA	Station marc delorme, Centre National de la Recherche Agronomique (Côte d'Ivoire)
CIB	Coconut industry board (Jamaica)
CICY	Centro de investigación Científica de Yucatan
Fadcanic	Fundación para la Autonomía y el Desarrollo de la Costa Atlántica de Nicaragua (Nicaragua)

## Introduction

The coconut cultivar Panama Tall (PNT) is the pollen parent of the MAYPAN hybrid, which has been planted extensively in Jamaica during the last 25 years, as a control measure against an epidemic disease, the Lethal Yellowing (LY) caused by a phytoplasma. In the late 70s, the results of resistance trials (Been 1981) had lead to encourage plantation of the Malayan Yellow Dwarf (MYD) and of the MYD × PNT hybrid or MAYPAN as a control measure against the disease. The PNT itself was not resistant: its mortality rate was 38–67% at 10 years according to the trial site (0–10% in the Malayan Dwarfs). The MAYPAN was the best hybrid with 4 and 21% mortality and was considered as a good compromise

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between resistance level, yield and product quality. Although it has fostered a revival of the Jamaican coconut industry for 20 years, this hybrid is now being massively destroyed by a new outbreak of the disease (Broschat et al. 2002).

A previous paper (Lebrun et al. 2007), demonstrated that the MYD was more variable in Jamaica than in the rest of the world, providing evidence of genetic contamination. However, this contamination appears to have existed since the introduction of the cultivar and thus is unlikely to be the main cause of the apparent loss of resistance since the 80s. We now consider the case of the other parent of the hybrid: the PNT.

The methods required to study the PNT differ from those used in the case of the MYD. Unlike the Dwarfs, Tall coconuts are predominantly cross-pollinating. As a result, all its individuals differ from each other although to a lesser extent than from individuals from other cultivars. Identifying members of cross-pollinating cultivars requires the use of probabilistic methods (Rannala and Mountain 1997). These methods have been developed for coconut as part of a microsatellite kit for coconut cultivar identification (Baudouin and Lebrun 2002) based on a set of 14 microsatellite markers. Diversity studies based on these markers and conducted on a large number of coconut cultivars made it possible to distinguish two main genetic groups: Indo-Atlantic and Pacific (Lebrun et al. 2003).

We show here how assignment methods can be used to assess the trueness-to-type of genetic material used in genetic trials and in seed production. All tested samples in the present study are supposed to be PNTs, but unwanted intercrossing may have occurred during rejuvenations. Apart from the PNT, which originated in the Pacific coast of Panama, two cultivars are widespread in Jamaica and are thus the most probable sources of genetic contamination. The local cultivar is the Jamaica Tall (JMT) and belongs to the Indo-Atlantic group. Quite similar coconut cultivars are widespread in the whole Caribbean region including the Atlantic coast of Mexico and Panama where they are known as Alto Atlantico. The Malayan Dwarfs (mainly MYDs) was introduced several times during the last century. It belongs to the Pacific group, like the PNT, but these cultivars are easy to distinguish, thank to their low diversity. As a result, it is often possible to identify the source of contaminating pollen

and the type of cross and to assess its potential effect on LY resistance level. This paper presents the characterization of 86 PNT individuals from four populations in Jamaica. These populations have been used as pollen sources for seed production since the 70s.

## Materials and methods

### The Microsatellite kit

The molecular markers used in this study were 14 microsatellite (SSR) markers from the “microsatellite kit for coconut cultivar identification” (Baudouin and Lebrun 2002; Lebrun et al. 2005). The selection criteria for these markers were that they should be easy to implement and to score. SSR analyses were performed on an automatic sequencer Li-Cor IR2 (Lincoln, Nebraska). All technical conditions are described in Baudouin et al. (2006).

### Reference populations

We used six representative samples (Table 1) from populations already characterized in previous studies (Lebrun et al. 2005) as reference populations for the three main cultivars found in Jamaica:

- In order to represent the diversity of the PNT, we considered three sub-populations of this cultivar: the first one corresponds to what can be considered as the “typical” Panama Tall (PNTty); although the sample is made up of individuals collected at two different places, it is very homogeneous. The second one comes from two fields planted with the same population “Aguadulce” (PNTagu). About 10% of its genes result from introgression from “Alto Atlantico”. The third one (PNTcr, from Costa Rica) can only be distinguished from the “typical” PNTs by the presence of a small number of specific alleles.
- We represented the MYD by 69 individuals, representative of the local population and already studied in Lebrun et al. (2007). The local population is more variable than the MYD found elsewhere in the world, which is usually monomorphic.
- Finally, we represented the JMT by two samples, one originating from the JMT itself and one made up of the closely related Mexican Atlantic Tall (MXAT).



**Table 1** Origin of the reference samples

Cultivar	Reference sample	Size	Origin	Provided by
Panama Tall: PNT (group A7) cross-pollinating	"Typical" PNT (PNTty)	26	22 from Monagre (Panama) 4 from Peru	SMD-CNRA CIB
	PNT Aguadulce (PNTagu)	27	14 from Aguadulce (Panama) 13 from Nicaragua	SMD-CNRA Fadcanic
	PNT Costa Rica (PNTcr)	21	21 from Costa Rica	Fadcanic
Malayan Yellow Dwarf: MYD (group A1a) self-pollinating	Local MYD (MYDjam)	69	5 from Fair prospects 43 from Barton Isle 11 from Hemitage 10 from Ballard Valley	CIB
	Jamaica Tall (JMT)	5	Jamaica	CIB
	(group B1) cross-pollinating Mexican Atlantic Tall (MXAT)	9	Campeche (Mexico)	CICY

### Tested material

The tested material consisted in 86 genotypes collected at four locations. Ten genotypes (noted BD) came from Bowden. The samples from Green Castle and were divided into four batches based on the colour of their inflorescences: three groups of ten trees each with green (GCa), bronze (GCc) and intermediate (GCb) inflorescence. The colour was not specified for the last batch (GC). Twenty-six individuals came from Plantain Gardens: 15 of them (PGa) had green inflorescences and 11 (PGc) had bronze inflorescences. Finally, 11 samples from Agualta Vale were noted "AV".

### Statistical analyses

We used software *GeneClass 2* (Piry et al. 2004) to calculate the assignment scores of the reference populations for all PNT individuals. This score is equal to  $-\log_{10} L_{ij}$ , where  $L_{ij}$  is the likelihood of population  $i$  for individual  $j$ . The more a population is likely to be the source of an individual, the lower is its score for this individual. Absolute values are almost meaningless because they depend on the number of loci and of their discriminating values. However, comparison between populations for the same individual is meaningful: the most probable origin of the individual is the population with the lowest score (Baudouin et al. 2004). Since the score is logarithmic, a difference of, e.g. 3, 6 or 9 means that likelihood ratio are, respectively, 1,000,  $10^6$  or  $10^9$ .

Likewise, comparisons between individuals can help detecting possible hybrids: the presence of genes from population B in a member of population A is expected to increase the score of population A and to reduce that of population B. It will also increase its heterozygosity. Although none of these criteria is sufficient by itself to consider an individual as an outlier, their combination amounts to a strong suspicion of hybridization. We were thus able to identify probable outliers and, in some cases, to formulate hypotheses about their true origin (e.g. a  $F_1$  hybrid between PNT and another cultivar). Finally, we confirmed these hypotheses by direct examination of their genotypes.

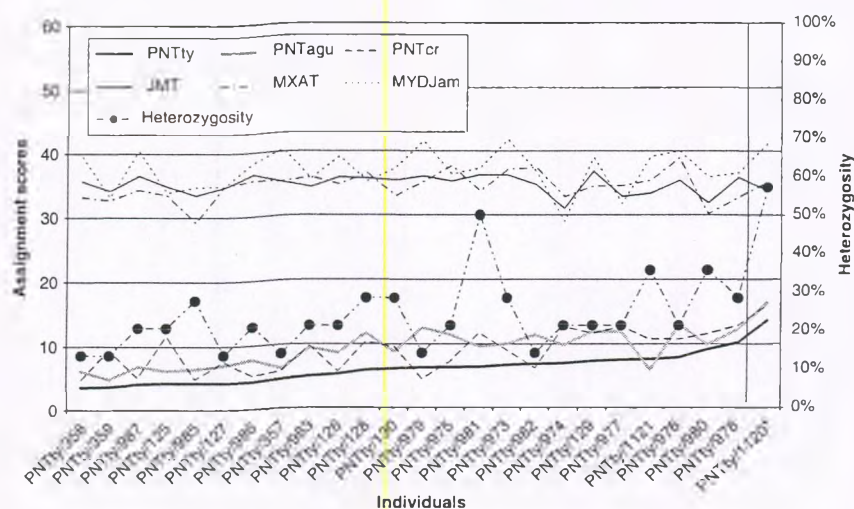
To validate our findings, we also ran *Structure 2.1* software (Falush et al. 2003) using population information for the reference populations only and allowing for admixture. A 10,000 iteration period was followed by 50,000 iterations.

## Results

### Reference populations

In order to calibrate the hybridization criteria, we determined the normal variation range of heterozygosity and of assignment scores among PNT of known origin. Figure 1 represents the results for the "typical" PNTs. We ordered individuals by increasing score for their population of origin. For all but the last one, the score associated to the "typical" PNT varied smoothly from 3 to 10. Scores for the Aguadulce and

**Fig. 1** Assignment scores and heterozygosity in the true Panama Tall. Individuals were ordered according to the score for the typical Panama Talls. The trueness-to-type of individual PNTy1120 (to the right) is dubious. It was excluded from subsequent analyses



Costa Rica populations were in average three units above the “typical” sub-population, which is just enough to ensure that most “typical” PNTs are assigned to their true origin, rather than to one of the other sub-populations of the PNT. Contrastingly, the scores of the JMT, MXAT and MYD cultivars were between 30 and 40 preventing any confusion with these cultivars. Finally, heterozygosity was generally comprised between 0.15 and 0.35. Only individual PNTy/1120 could be seen as suspect: its genotype was 22,000 times less probable than the next least probable and its heterozygosity was unusually high. We removed this individual from subsequent analyses, although its influence on the results is negligible.

The results for the members of the other PNT sub-populations (Aguadulce and Costa Rica—not shown) were quite similar and the scores of their respective origins were also comprised between 3 and 10 and only slightly higher for the other Panamean populations. The scores of JMT and MXAT were comprised between 30 and 45. Heterozygosity was more variable and appreciably larger in the Aguadulce population (0.38 in average instead of 0.25 and 0.29 for PNTy and PNTcr, respectively).

To summarize this section devoted to the reference samples, a PNT has a score ranging between 3 and 10 for its own source sub-population. Differences among PNT origins result in a slight increase of the scores of the other sub-populations. On the other hand, confusion with the Indo-Atlantic Talls or the MYD is impossible with scores systematically exceeding 25. In addition, a heterozygosity rate

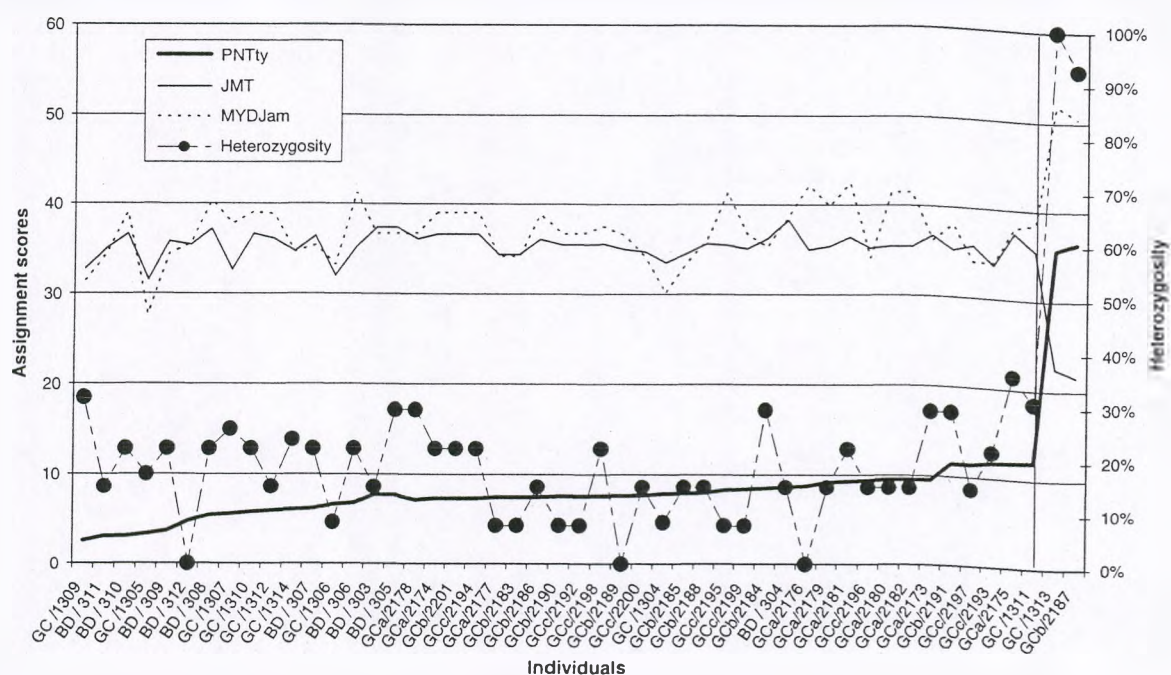
above 0.25 is unusual, except for the Aguadulce origin. We could thus set the following thresholds for the present study: if an individual had a score below 12 for the PNTy, we considered it as true-to-type. If its score was above 20 for the PNTy and below 25 for one of the possible source of contamination, we considered it as an outlier. In between, its trueness to type was dubious, especially if its heterozygosity was high.

#### Tested populations

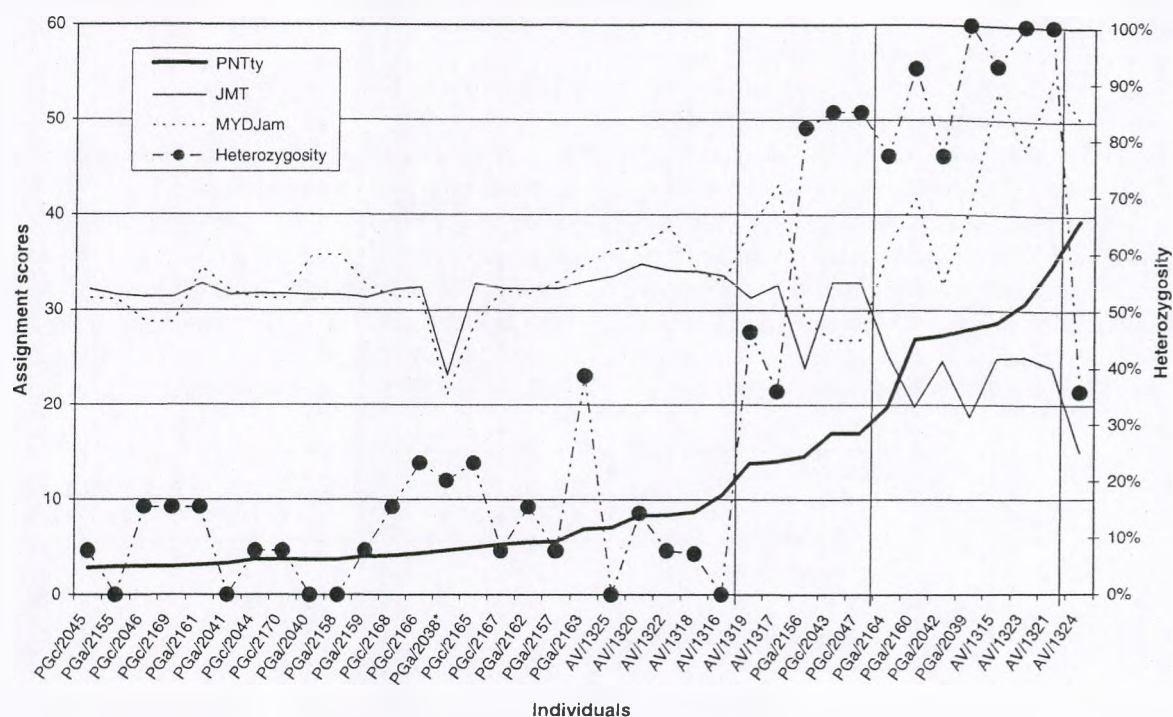
Figure 2 represents the results of the PNT planted at Green Castle and Bowden. They behaved very much like the Panama reference populations, except for individuals GC/1313 and GCb/2187, which we identified as  $F_1$  hybrids between PNT and JMT: they both combine a high score for the PNT and the MYD with a low score for the JMT and high heterozygosity. Although the colour of the inflorescence is of genetic origin and is potentially a diagnostic criterion, we did not find differences between colours: the mean scores were, respectively, 8.9, 8.1 and 8.7 for categories GCa, GCb and GCc.

Figure 3 represents the results at Agualta Vale and Plantain Gardens. Four groups can be distinguished: 24 individuals were true PNTs (left hand part of the graph). Putative hybrids (right hand part) were represented by a first generation back cross with JMT (AV/1324) and seven  $F_1$  hybrids between PNT and JMT. Finally, five individuals were of dubious origin: with a score comprised between 12 and 20, they are proba-





**Fig. 2** Assignment scores and heterozygosity at Green Castle and Bowden. For clarity, score for Aguadulce, Costa Rica and MXAT are not shown. All individuals are conform except for GC /1313 and CCb/2167 (to the right) which are PNT  $\times$  JMT  $F_1$  hybrids



**Fig. 3** Assignment scores and heterozygosity at Agualta Vale, and Plantain Gardens. For clarity, score for Aguadulce, Costa Rica and MXAT are not shown. From the left to the right: 24 truly Panama Talls (the abnormal scores for individual pga/2038

are due to missing values); five individuals of dubious origin; seven PNT  $\times$  JMT  $F_1$  hybrids and one (PNT  $\times$  JMT)  $\times$  JMT  $BC_1$  hybrid

**Table 2** Summary results of the tested material

	Number of palms	True Panama Tall	Dubious	Hybrids with JMT			
				F1		BC1	
Theoretical percentage of Panama Tall genes		100%	n. a.	50%		25%	
Bowden							
	10	10	100%				
Green castle							
Unspecified colour	10	9	90%	1	10%		
A (green)	10	10	100%				
B (intermediate)	10	9	90%	1	10%		
C (bronze)	9	9	100%				
Average	39	37	95%	2	5%		
Plantain garden							
A (green)	15	10	67%	3	20%	2	13%
C (bronze)	11	9	82%	2	18%		
Average	26	19	73%	5	19%	2	8%
Agualta Vale							
	11	5	45%	2	18%	3	27%
Grand total	86	71	83%	7	8%	7	6%
						1	9
						1	1

bly neither F1 hybrids, nor pure PNTs. These results are summarized in Table 2.

Finally, we note that, considering only the “true to type” individuals, observed heterozygosity was somewhat lower in the Jamaican populations (12–18%) than in the “typical” PNT (23%). As a result, nine individuals were totally homozygous: one in Bowden, two in Green Castle, four in Plantain Garden and two in Agualta Vale. This can be explained by several factors, including variation among the PNT populations collected initially, varying sample sizes and the fact that some of Jamaican populations represented the second or third generation since introduction, result-

ing in a lower genetic diversity, compared to the reference sample, which was a direct introduction.

#### Comparison with *Structure* software

We ran *Structure* 2.1 six times for each value of the assumed population number ranging from 3 to 6. A stable and minimal value of the log-likelihood was obtained only for five populations, which could be identified as the three PNT populations, the MYD from Jamaica and a last population grouping the JMT and the MXAT. The population parameters are given in Table 3. The estimated migration rate  $\alpha_1$  was low

**Table 3** Summary results of *Structure*

Migration rate	$\alpha_1 = 0.047$				
	PNTty	PNTagu	PNTcr	MYDJam	JMT + MXAT
Population parameters					
$F_{ST}$	0.43	0.34	0.37	0.48	0.31
Kullback-Leibler “distance”					
PNTty	–	0.43	0.41	2.76	2.28
PNTagu	0.72	–	0.76	2.76	1.73
PNTcr	0.48	0.60	–	3.00	2.26
MYDJam	3.82	2.68	2.94	–	2.70
JMT + MXAT	4.61	2.55	3.36	3.86	–



and confirmed that the reference populations were clearly differentiated. However, the Kullback-Leibler “distances” among the three PNT sub-populations were much lower than the others (Table 3), confirming their close relationship. The estimated percentage of genes from JMT was 8% at Plantain Gardens and 26% at Aqualta Vale.

Individuals identified as “true” PNTs based on the above criteria were confirmed by *Structure*, with an estimated percentage of Panamean genes above 97%. This percentage was comprised between 60 and 90% for the “dubious” genotypes and the “off-type” genotypes were also confirmed with a percentage of genes from the parental populations close to theoretical values for first or second generation hybrids. According to *Structure*, only three trees (PGc/ 2043, PGc/2047 and PGa/2042, see Fig. 3) inherited an appreciable part of their genes from the MYD. Minor differences of appreciation between the two approaches were noted in the case of GC/1313 and GCb/2187 (see Fig. 2): *Structure* suggest they are BC<sub>1</sub> towards JMT. Their high heterozygosity, among other things, indicate that they are actually F<sub>1</sub> hybrids.

## Conclusion

The assignment method implemented in *GeneClass 2* was primarily devised to assign individuals to pre-determined populations in cross-pollinating species. This paper illustrates its use to identify off-types among the members of a population. Both *GeneClass 2* and *Structure 2.1* have their own advantages: *GeneClass 2* does not use the MCMC procedure and the result was obtained in a matter of seconds. *Structure 2.1* was significantly more difficult to handle: we had to try several parameter combinations before obtaining stable and reliable results, but directly estimated the proportion of genes coming from the parent populations, confirming most of our hypotheses on the hybrids. Finally, the benefit of using two very different algorithms was to give us more confidence in our results.

We demonstrated the presence of alleles from other populations in all the studied PNT populations, except for Bowden. At Green Castle, only two palms were F<sub>1</sub> hybrids, while in Aqualta Vale and Plantain Gardens, 30–55% were not pure PNTs. This means that the controlled pollination techniques used for

producing the last two accessions were not always perfect and that care should be taken in the future to maintain the genuine PNT. This implies to use palms known to be true to type and to apply an efficient controlled pollination method, such as those recommended in Santos et al. (1996).

The main source of non-PNT alleles was the JMT and seed production using pollen from Aqualta Vale and Plantain Gardens is likely to result in an increased susceptibility to LY. Actually, these seed gardens contributed little to seed production in Jamaica: in the 80s, pollen came from St. James and White Hall Estates. In the early 90s, it came from Bowden and later on, Green Castle represented the main source, with an approximate 1% contribution from Plantain Gardens. Since St. James and White Hall Estates have been wiped out by the disease, their status is unknown. One may however suspect that, due to their age, they had less chance to be contaminated by unwanted pollen than more recent ones. Bowden was also destroyed since the sample collection.

Despite uncertainties about the status of some of the pollen sources, genetic contamination in the PNT was probably not the main reason for the increased mortality in the MAYPAN: firstly, this cultivar has never been fully resistant: its mortality rate ranged from 4 to 21% in Been (1981). Secondly, the most contaminated pollen sources do not appear to have been used in large quantities. The presence of up to 26% of JMT genes in a number of pollen batches was certainly sufficient to increase substantially mortality in a few plantations, but not to cause the “massive losses” that have been noted by Broschat et al. (2002). We have thus to conclude that neither the MAYPAN, nor its parents can be said truly and permanently resistant.

True or apparent variations of resistance level in these cultivars may have occurred for several reasons: mutation and selection in the phytoplasma may have favoured virulence against the MYD and the MAYPAN. On the other hand, the behaviour of the vector may also have changed: in the resistance trials, the MYD and the MAYPAN were planted together with many different cultivars. The vectors may have preferentially fed on other cultivars, such as the JMT, which were thus destroyed more rapidly. But, once the MAYPAN and the MYD represented the majority of the commercial plantations, they had no other

choice than feeding on these cultivars. In the first hypothesis, the MAYPAN had (incomplete) resistance factors, mainly inherited from the MYD and these resistance factors were progressively overcome during the last 25 years. In the second one, neither the MYD nor the MAYPAN were resistant, but they initially escaped the disease. In both hypotheses, the new outbreak was favoured by the extensive planting of two closely related cultivars, with a narrow genetic basis.

In the present state of knowledge, no truly and permanently resistant cultivar to the Jamaican form of LY is known. As a result, a strategy to control LY should probably rely on an increased diversity of the planting material, rather than on a small number of highly uniform varieties. In Been (1981), several cultivars had a reduced mortality rate (although higher than for the MYD). They generally came from South-East Asia. Planting several cultivars from this region would probably reduce the rate of evolution of the pathogen and/or the vector, owing to their increased diversity. It would also probably make the effects of this evolution less dramatic. Such measures could probably contribute to increasing the economic life of coconut plantations in Jamaica, and thus to restoring their profitability. It would however not make them free of LY: all treatments of the trials mentioned in Been (1981) are now severely affected by LY (B. Been, W. Myrie, not published data). More research is needed for a better understanding of the conditions that affect the transmission of the disease.

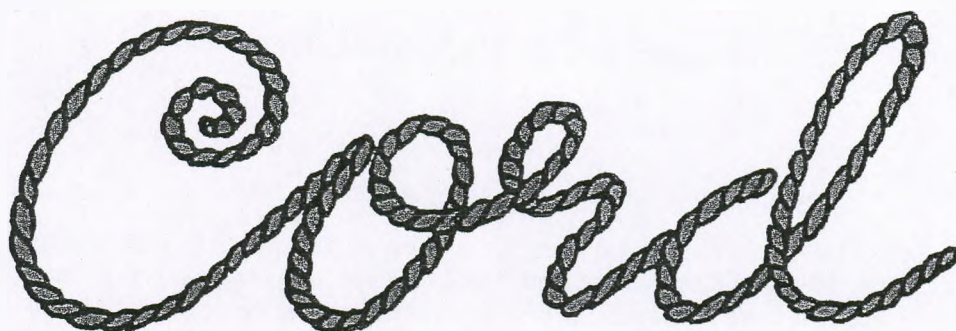
**Acknowledgements** We thank IPGRI and the Cogent network, which financed the development of the coconut cultivar identification kit, and the Genotyping Platform of the Languedoc Roussillon Genopole, hosted by Cirad (C. Billot and R. Rivallan) where all the SSR data have been obtained.

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## **Appendix 6**

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**Asian and Pacific Coconut Community**



## New data on a cultural control method against coconut lethal yellowing in Ghana

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### Abstract

Lethal yellowing disease has been rife in West Africa, especially Ghana, since 1932. In the 1990s, the first operations to fell diseased coconut palms at an early stage showed that this substantially slowed down the spread of the disease.

Trial conducted in 1995 showed that early felling of diseased coconut palms, even without prior treatment, considerably slowed down the spread of the disease. Replications of this action in several other plots kept them healthy for many years. These positive results made it possible to obtain funding from *Agence française de Développement* (AFD) to maintain a "sanitary cordon" in the far West of the Western Region of Ghana, near the Ivorian border, where there is a wide area of coconut palms.

**Keywords:** Coconut, lethal yellowing, early felling and Ghana.

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## Introduction

Coconut (*Cocos nucifera*) is a well-known cash crop in the coastal region of Ghana. It is a good source of income for numerous smallholders in the zone. It also generates jobs in this rural zone. Indeed, annual coconut production is estimated at several hundred thousand tonnes from 43,000 ha of coconut plantings (Ofori and Nkansah Poku, 1997).

A very serious disease, coconut lethal yellowing, caused by a phytoplasma (Tymon *et al.*, 1995) and probably transmitted by a sucking-piercing insect, has been rife for many years in Ghana, firstly in Volta Region, then Western Region and lastly Central Region. In all, throughout these three regions of Ghana, over 10,000 ha of coconut plantings have already been decimated and the disease is currently very active in the Central and Western Regions (Dery *et al.*, 1997).

After 15 to 20 years, trials of coconut hybrid varieties have revealed two coconut varieties with tolerance or resistance to this disease (Mariau *et al.*, 1996; Dery *et al.*, 1997 and 1999). A rehabilitation programme for Ghanaian coconut plantings is under way with funding from *Agence Francaise de Developpement (AFD)*.

In order to slow down the spread of this disease, which can be rapid in the rainy season, trials involving felling and insecticide treatment against sucking-piercing insects have been carried out in the Central Region of Ghana.

An early felling trial was conducted in 4 plots in the locality of Ayensudu (Central Region) beginning in 1995. Initial data were presented at the first international seminar held at Ceiba Atlantida (Honduras) on 16 and 17 November 2000 (Dery *et al.*, 2000). Observations continued after that date to determine the spread of the disease when felling operations were suddenly halted.

## Materials and methods

In the vicinity of Ayensudu (Central Region), the disease started to develop in four

plots: diseased coconut palms at all stages of development and different levels of infection according to the number of diseased coconut palms at the beginning of the trial were available (Table 1). It was thus possible to carry out the following interventions:

- Plot 21 (248 coconut palms): Felling only each time new disease cases occurred.
- Plot 22 (134 coconut palms): Felling + only 2 hot-fogging treatments were carried out at the beginning.

The first treatment was carried out on 22 June 1995 with Propoxur (Unden liquid 200 g *a.i.* per litre): 0.5 litres of Unden mixed with 6 litres of diesel oil. One mixture was hot-fogged in the plot. After that first treatment, two diseased coconut palms were felled the same day. Six months later, on 22 December 1995 a second treatment was carried out with chlorpyrifos (Durban 480 g *a.i.* per litre): 0.5 litres of Dursban mixed with 6 litres of diesel oil. One mixture was hot-fogged in the plot. No diseased coconut palms were observed at that time.

Felling was continued each time new disease cases appeared.

- Plot 23 (301 coconut palms): Felling + hot-fogging treatment with chlorpyrifos each time new cases occurred.

The first treatment was carried out on 28/12/95 with the same dosage of chlorpyrifos as above. Two mixtures were hot-fogged in the plot. After treatment, 6 diseased coconut palms were felled the same day. The second treatment was carried out roughly one year after the first one, on 15/10/96 with the same insecticide at the same dosage. After treatment, 3 diseased coconut palms were felled the same day. The third treatment was carried out 1.5 years after the second one, on 28/03/98 with the same insecticide at the same dosage. After treatment, 2 diseased coconut palms were felled the same day. Thereafter, felling was continued in the plot each time new cases occurred.

- Plot 24 (360 coconut palms): only one hot-fogging treatment without felling was carried out on

23/07/96 with chlorpyrifos. Three mixtures of Durban with diesel oil, as above, were hot-fogged in the plot. There were already 14 diseased coconut palms, which were not felled (Table 1).

Checks were made every three months and physical or chemical action was taken, where necessary, in the week following the observations.

### Results and discussions

Felling of the first diseased coconuts showing the very first symptoms of infection (nut fall and yellowing of lower fronds), appreciably reduced disease extension within an infected plot. Over a 5-year period (1995-1999), losses on coconut farms varied from 3 to 4% (Table 1). However, the difference with the effect of felling combined with hot-fogging treatments was not significant. Furthermore, very early intervention in a plot with fewer than five diseased coconuts prevented intensive multiplication of the infectious inoculums.

Figure 1 shows that the coconut palms rapidly became infected once all the felling and treatment operations were stopped.

Near the village of Abakrampa, a coconut planting of around 14 ha was infected in December 1992 (near the Secondary School = 1 coconut palm infected; near the village = 2 coconut palms infected). Felling was carried out in May 1993. In July 1995, 7 new diseased coconut palms were detected near the village and were felled in September 95. In July 1996, another 7 cases were identified near the village and were felled a month later. Felling was then halted because of lack of funds; the disease gradually eliminated the palm grove within 5 years.

It has been further demonstrated in several villages: Azuleti, Moree, that when felling is carried out right at the beginning of a disease focus (1 to 3 coconuts affected) the spread is drastically slowed down. For example, at Azuleti, felling was carried out in 1995. To date, the

disease has not reappeared. In some instances, further felling is required. Where this is not done or action is delayed, the disease spreads very rapidly (e.g. Asebu).

These results indicate that felling reduces the inoculums to such a low level that the occurrence of new disease cases is distinctly slowed down. To date, disease transmission has not been formally elucidated, despite 4 years of experiments under an EC-funded STD3 project (Dery *et al.*, 1995a, 1996). Should transmission be by insects, these positive early felling results would suggest that the emergence of the disease in a healthy spot is only due to a few infectious sucking-piercing insects. So far, no outbreak of a particular species of this group of insects has ever been observed on diseased coconut palms isolated at the start of infection in a given plot. The first diseased coconut palms are several dozen metres apart and up to a few hundred metres apart in the same infected plot. At the start of the disease in a plot, it is rare to see two neighbouring infected palms. However, if the first diseased palms are left in place, neighbouring palms can be contaminated in turn by any sucking-piercing insects capable of transporting the pathogen. The time taken for transmission from the first infected coconut palms to their neighbours would therefore seem to depend on the speed with which the phytoplasma multiplies in the plant, and the number of sucking-piercing insects in the immediate vicinity of the first coconut palms infected.

If there are more than 10 coconut palms showing symptoms of the disease, felling will no longer give the same beneficial effect. It would seem that by this level of infection, there is sufficient inoculum for the disease to be spread rapidly by sucking-piercing insects. Palms subsequently infected are the immediate neighbours of the first infected palms, and the disease spreads in "patches" (Dery and Philippe, 1995b). Consequently, it would be useful to start felling as soon as diseased coconut palms appear, so as to prevent the inoculum from accumulating in a given plot.



**Table 1. Effect of a felling and/or chemical treatment on the spread of coconut lethal yellowing**

Treatments	Total number of coconut palms affected by lethal yellowing									
	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
Plot 2i (248 coconut palms) Felling only	1 (0.4%)	2 (0.8%)	2 (0.8%)	4 (1.6%)	8 (3%)	27 (11%)	62 (25%)	190 (76.6%)	228 (91.9%)	248 (100%)
Plot 22 (134 coconut palms) Felling + 2 treatments only in 1995	2 (1.5%)	2 (1.5%)	2 (1.5%)	2 (1.5%)	4 (3%)	19 (14%)	48 (35.8%)	98 (73.1%)	123 (91.8%)	131 (97.8%)
Plot 23 (301 coconut palms) Felling + Treatment as new cases occurred (3 treatments)	6 (2%)	9 (3%)	9 (3%)	11 (3.65%)	11 (3.65%)	18 (6%)	40 (13.3%)	224 (74.4%)	282 (93.7%)	297 (98.7%)
Plot 24 (360 coconut palms) One treatment only without cutting all along the trial		14 (4%)	70 (19%)	160 (44%)	285 (79%)	296 (82%)	298 (82.7%)	299 (83%)	359 (99.7%)	359 (99.7%)



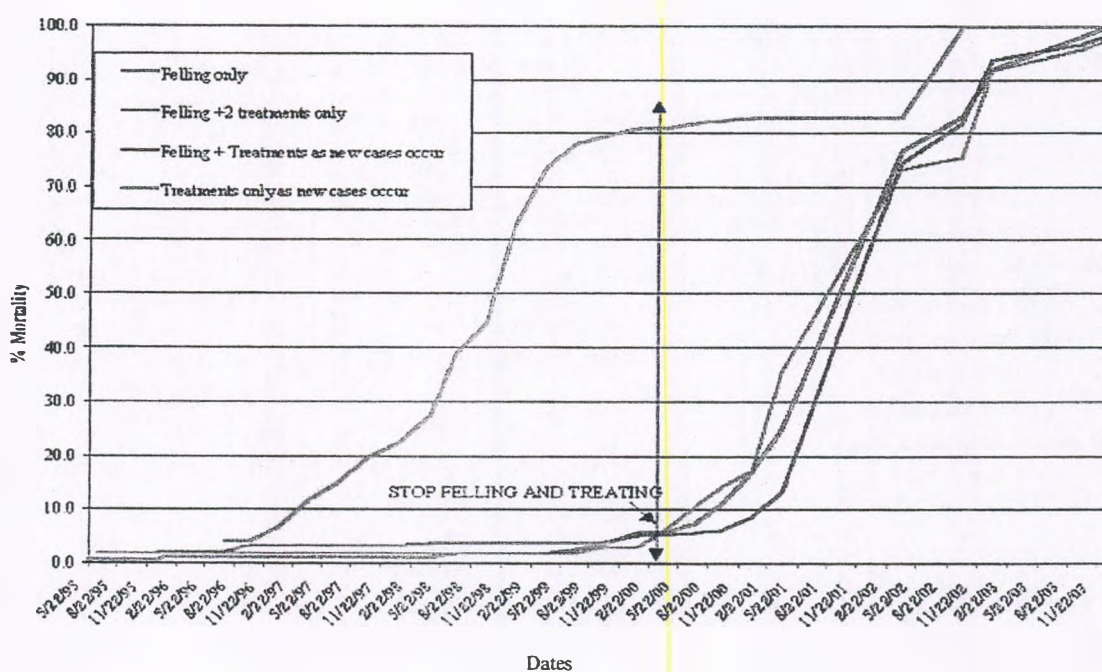


Fig. 1. Effect of felling (with or without treatment) on the spread of coconut lethal yellowing

Under a project funded by AFD, early felling or containment operations are being carried out from April 2003 by a specialized team (3 people) equipped with a pick-up truck and a chainsaw. The main purpose of this dedicated team is to prevent any disease propagation in the extreme West of Ghana, which is covered by a large area of coconut palms (around 18,000 ha in one swathe belonging to around 15,000 growers). In that part of Western Region, coconut is the main cash crop. Thus, any new disease foci at Nkroful (Western Region) for example have been eradicated as and when they appear. The same applies for old foci at Ampain (Western Region). These two foci were regularly monitored and sanitized. A total of 434 diseased palms were eliminated at these foci: 175 at Ampain and 259 at Nkroful (Table 2). Another disease front in the Shama Ahanta East District was also contained: 94 diseased palms were felled at this focus. Two extensive surveys were carried in the Nzema East and Jomoro districts to identify any incipient disease focus. None was found beyond the Ampain focus. Beyond the main Nkroful focus, ten incipient foci were identified in the second survey conducted in October 2003. These were located at Teleku-Bobazo and the surrounding area. All the diseased palms in the foci were eliminated (Table 2). One other focus was also identified at Nkroful, near to the main focus.

**Table 2. Elimination of infected palms at identified lethal yellowing disease foci**

Location	No. of foci	No. of infected palms
Ampain	1	175
Nkroful	2	259
Teleku-Bokazo	10	169
Dabose Junction	1	94
Total	14	697

This method has apparently been applied in Mozambique (AFD project) in the Madal coconut estate with success. It has yet to be applied to

smallholdings (de Franqueville, 2004, personal communication).

Felling infected coconut palms is a method that has also been applied to a coconut root disease (Coconut root (wilt) disease) in Kerala (India), which is apparently caused by a phytoplasma (Solomon *et al.*, 1983). This disease does not kill the palms, but it causes a considerable drop in yields (Rethinam *et al.*, 1982). In this case, felling is followed by phytosanitary measures, unlike with lethal yellowing where diseased palms are felled early and left as they are in the plots. After felling, palms with infected roots are burnt on site. Carbaryl treatments are carried out to eliminate the vector *Stephanitis typica* (Muralidharan *et al.*, 1990). All in all, a code of conduct has been adopted by the official authorities in Kerala: identification of all diseased coconut palms, spraying with 0.05% carbaryl, felling and removal of infected palms with their bole and roots, and burning of the bole and roots on site.

### Conclusion

It may be possible for growers to proceed solely with early felling of diseased coconut palms revealed by meticulous monitoring of their coconut plantings. Under no circumstances felling should be delayed once there are more than 5 diseased coconut palms, otherwise felling would seem no longer to slow down the spread of the disease. In that way, early felling of palms showing symptoms of the disease should allow growers to avoid the additional work required for chemical treatments.

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